



## Case report

Atypical phenotype in two patients with *LAMA2* mutations

Joana Marques<sup>a,\*,1</sup>, Sofia T. Duarte<sup>b,c,1</sup>, Sónia Costa<sup>d</sup>, Sandra Jacinto<sup>b,c</sup>, Jorge Oliveira<sup>e</sup>,  
 Márcia E. Oliveira<sup>e</sup>, Rosário Santos<sup>e,f</sup>, Elsa Bronze-da-Rocha<sup>f</sup>, Ana Rita Silvestre<sup>g</sup>,  
 Eulália Calado<sup>c</sup>, Teresinha Evangelista<sup>b,g</sup>

<sup>a</sup> Serviço de Neurologia, Instituto Português de Oncologia de Lisboa, Francisco Gentil, Rua Professor Lima Basto, 1099-023 Lisboa, Portugal

<sup>b</sup> Instituto de Medicina Molecular, Faculdade de Medicina da Universidade de Lisboa, Av. Professor Egas Moniz, 1649-028 Lisboa, Portugal

<sup>c</sup> Serviço de Neurologia Pediátrica, Hospital D. Estefânia, Centro Hospitalar Lisboa Central, Rua Jacinta Marto, 1169-045 Lisboa, Portugal

<sup>d</sup> Serviço de Neurologia, Hospital Vila Franca de Xira, Estrada Nacional No. 1, Povos, 2600-009 Vila Franca de Xira, Portugal

<sup>e</sup> Unidade de Genética Molecular, Centro de Genética Médica Dr. Jacinto Magalhães, Centro Hospitalar do Porto, Praça Pedro Nunes, No. 88, 4099-028 Porto, Portugal

<sup>f</sup> Departamento de Ciências Biológicas, Laboratório de Bioquímica, Faculdade de Farmácia da Universidade do Porto, Rua de Jorge Viterbo Ferreira, No. 228, 4050-313 Porto, Portugal

<sup>g</sup> Laboratório de Neuropatologia, Serviço de Neurologia, Hospital de Santa Maria, CHLN, Av. Professor Egas Moniz, 1649-035 Lisboa, Portugal

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## Abstract

Congenital muscular dystrophy type 1A is caused by mutations in the *LAMA2* gene, which encodes the  $\alpha 2$ -chain of laminin. We report two patients with partial laminin- $\alpha 2$  deficiency and atypical phenotypes, one with almost exclusive central nervous system involvement (cognitive impairment and refractory epilepsy) and the second with marked cardiac dysfunction, rigid spine syndrome and limb-girdle weakness. Patients underwent clinical, histopathological, imaging and genetic studies. Both cases have two heterozygous *LAMA2* variants sharing a potentially pathogenic missense mutation c.2461A>C (p.Thr821Pro) located in exon 18. Brain MRI was instrumental for the diagnosis, since muscular examination and motor achievements were normal in the first patient and there was a severe cardiac involvement in the second. The clinical phenotype of the patients is markedly different which could in part be explained by the different combination of mutations types (two missense versus a missense and a truncating mutation).

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## 1. Introduction

Mutations in *LAMA2*, encoding the  $\alpha 2$ -subunit of the extracellular matrix protein laminin, are normally associated with muscular dystrophy and white matter abnormalities of the central nervous system (CNS).

Classical congenital muscular dystrophy type 1A (MDC1A) is characterized by severe generalized muscle weakness, joint contractures and peripheral neuropathy [1]. Primary partial laminin- $\alpha 2$  deficiency is associated with a less severe phenotype and with clinical and genetic heterogeneity [2–4].

In cardiac muscle, through its interactions with the  $\alpha 2$ -chain of laminin 211,  $\alpha$ -dystroglycan makes an important connection to the extracellular matrix, forming a link between the sarcolemma and the

\* Corresponding author. Tel.: +351 963113949; fax: +351 217229880.

E-mail address: [joanarlmrmarques@gmail.com](mailto:joanarlmrmarques@gmail.com) (J. Marques).

<sup>1</sup> Both authors contributed equally to this work.

extracellular matrix [5]. Symptomatic and subclinical cardiac involvement has been previously reported, notably in typical cases with absent laminin- $\alpha 2$  staining [4,6,7]. Regarding partial defects, cardiac involvement was documented only in one patient [8].

Previous studies indicate that laminin deficiency delays oligodendrocyte maturation through dysregulation of critical developmental signaling pathways [9]. Laminin- $\alpha 2$  binds to  $\beta 1$ -integrin and is distributed punctately on cortical neuronal processes [10]. Cerebral white-matter changes are typical; cortical dysplasia and polymicrogyria are seldom seen. Most patients are cognitively normal, but epilepsy and mental retardation have been described [11].

We report two patients with atypical phenotypes for primary partial laminin- $\alpha 2$  deficiency. The first case is remarkable for the predominant CNS involvement with normal muscular strength and the second had an unusually severe cardiac involvement.

## 2. Case reports

### 2.1. Patient 1

This patient is the second child of healthy unrelated parents and he was born after an uneventful

pregnancy. Macrocephaly (3SD) was detected in the first year of life. Refractory epilepsy associated with progressive cognitive regression started by the age of 6, with atypical absences, myoclonic and atonic seizures. Physical exam revealed macrocephaly and bilateral divergent strabismus. Motor complaints were never reported. He had normal muscular strength, tone and deep tendon reflexes. His gait was normal, with preserved coordination, and he was able to run, climb stairs and ride his bike. Brain magnetic resonance imaging (MRI) showed an area of agyria in the occipital cortex. In T2-weighted images, extensive white matter abnormalities, swelling and widening of gyri, mainly frontal, were detected (Fig. 1A and B). Serial electroencephalograms (EEG) revealed bilateral multifocal paroxysmal activity, suggestive of symptomatic epilepsy (Fig. 1C). *LAMA2* gene study was performed after extensive metabolic and genetic workup, given the occipital agyria and typical white matter abnormalities [12]. The highest creatine kinase (CK) value detected was 1589 UI/L. Electromyography (EMG), nerve conduction study and echocardiogram were unremarkable.

Epilepsy was initially controlled, but rapidly became refractory, with progressive cognitive impairment and

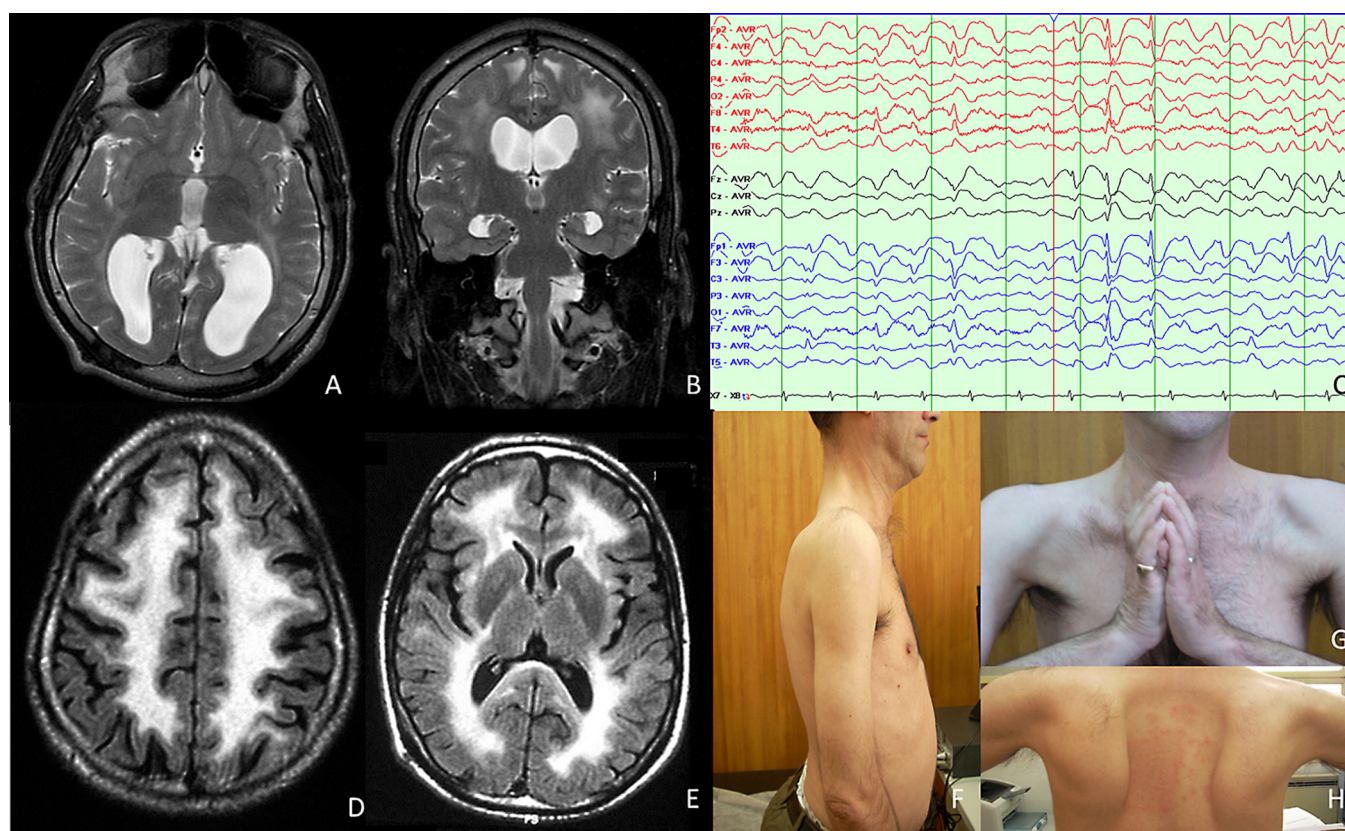


Fig. 1. T2-weighted axial (A) and coronal (B) brain MRI of patient 1 at age 15; note the occipital agyria and white matter hyperintensity; (C) EEG of patient 1: diffuse slowing of background activity; bilateral paroxysms, stronger in the anterior regions. (D and E) Axial FLAIR T2-weighted brain MRI of patient 2 with extensive white matter hyperintensities; (F) lumbar hyperlordosis in patient 2; (G) tendinous retractions in patient 2; (H) scapular winging in patient 2.

loss of expressive language. Motor function remains normal at age 21. Rufinamide significantly reduced the severity and frequency of atonic seizures.

## 2.2. Patient 2

This 55 year-old male patient was a single child of a healthy, non-consanguineous couple without family history of neuromuscular diseases. He presented delayed developmental motor milestones: he started walking after the age of 2 and always had difficulty running. By the age of 47 he sought medical attention, complaining of slowly progressive proximal muscle weakness and frequent falls, starting in his early forties. His neurological exam was notable for shoulder and pelvic girdle weakness, weak knee flexion and extension, and positive Gowers' maneuver; muscular atrophy with scapular winging (Fig. 1H); lumbar hyperlordosis (Fig. 1F) and dorsolumbar scoliosis. A rigid spine syndrome was noted as well as multiple tendinous retractions (Fig. 1G). Brain MRI showed typical white matter abnormalities (Fig. 1D and E). CK values were 351 UI/L. EMG was consistent with a myopathic process. Echocardiogram showed impaired left ventricular contractility and mitral valve prolapse. ECG Holter was normal. A slowly but progressive worsening of his motor function was noted over the years; he remains ambulant with bilateral support. Cardiac function has clearly declined and he developed dilated cardiomyopathy with dysfunction of the left ventricle contractility (shortening fraction 18%), marked dilatation

of the left ventricle and congestive heart failure NYHA class II–III.

## 3. Muscle histology

Specimens were obtained by open left deltoid muscle biopsy, frozen and stained using standard techniques. Additionally, 6  $\mu$ m sections were immunolabeled with antibodies targeting the 300 kDa N-terminal fragment of laminin- $\alpha$ 2 (NCL-merosin, Novocastra). Histological analysis in both cases revealed unspecific signs of muscular dystrophy (Fig. 2A–D). Immunohistochemistry labeling for dystrophin, sarcoglycans and dysferlin was normal, but a partial and irregular labeling for laminin- $\alpha$ 2 was documented in both specimens (Fig. 2E–G).

## 4. Molecular studies

### 4.1. Genomic DNA analysis

*LAMA2* gene sequencing was performed according to Oliveira et al. [2], where all coding and adjacent intronic sequences were analyzed. Sequence variants were described according to the Human Genome Variation Society guidelines for mutation nomenclature (version 2.0) [13] and the reference sequence with accession NM\_000426.3. Two heterozygous missense mutations were identified in patient 1, namely c.812C>T (p.Thr271Ile) in exon 5 and c.2461A>C (p.Thr821Pro) in exon 18 (Fig. 3). Family studies confirmed that these were allelic in the patient. Heterozygosity was also found in patient 2, who presented the missense mutation

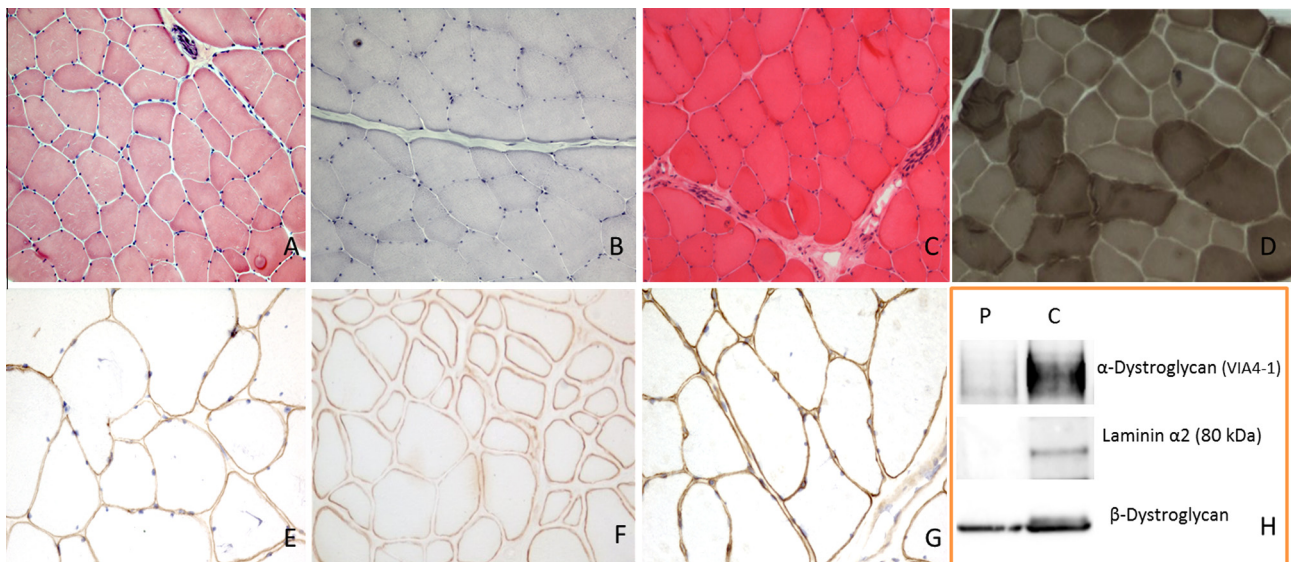


Fig. 2. Histologic and molecular studies performed in both patients. (A) Patient 1, hematoxylin and eosin staining (10 $\times$ ) discloses a mild increase in the variability of fiber diameter; (B) patient 1, gomori trichrome staining ( $\times$ 10); (C) patient 2, hematoxylin and eosin ( $\times$ 10), revealing increased variability of fiber diameter with round atrophic fibers, dispersed in the fascicles or in groups in the same area and frequent central nuclei; (D) patient 2, ATPase reaction (pH 9.4;  $\times$ 10). Type 1 fibers predominated; (E) partial and irregular laminin- $\alpha$ 2 labeling in patient 1 muscle specimen ( $\times$ 20); (F) partial and irregular laminin- $\alpha$ 2 labeling in patient 2 muscle specimen ( $\times$ 20); (G) normal control for laminin- $\alpha$ 2 labeling. (H) Immunoblotting analysis of patient 1 muscular sample by comparison to a control. P = patient; C = control muscle.



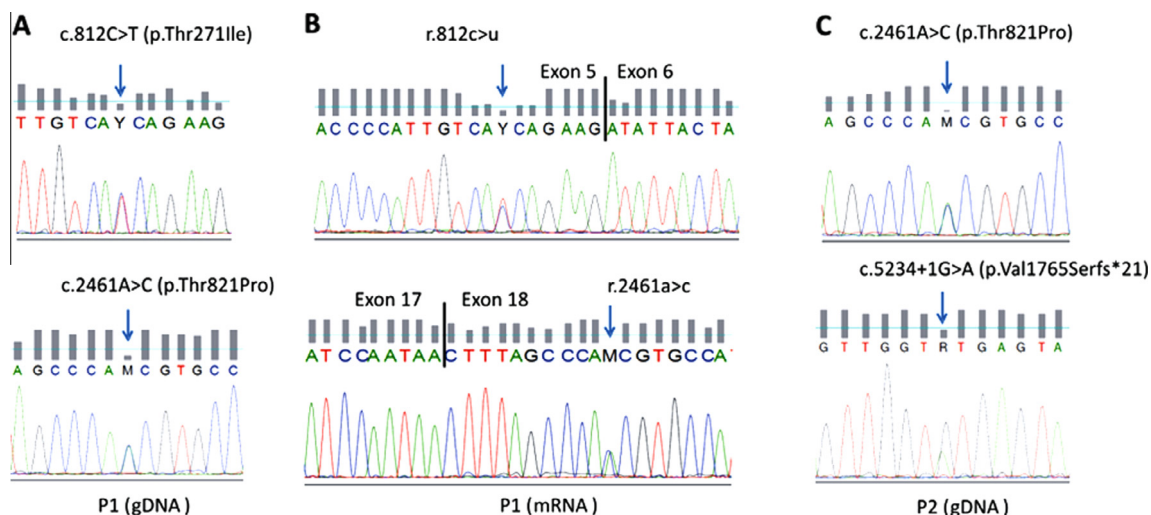


Fig. 3. Genetic studies performed in both patients. (A) gDNA sequencing results showing the two novel *LAMA2* mutations (arrows indicate changes) detected in heterozygosity in patient 1 (P1). (B) mRNA studies in patient 1, excluded possible splicing defects in the vicinity of the mutations. (C) Heterozygous mutations identified at the gDNA level in patient 2 (P2); the first in common with patient 1 and the second found to be a previously reported splicing mutation in intron 36 (c.5234+1G>A).

c.2461A>C (p.Thr821Pro), in common with patient 1, and the splicing mutation c.5234+1G>A (p.Val1765Serfs\*21) known to cause skipping of exon 36 [2].

Population screening for these missense variants was carried out by direct sequencing of exon 5 in the case of c.812C>T and by high resolution melting curve analysis (hrMCA) of exon 18 in the case of c.2461A>C. Neither variants were detected in 300 control alleles, corroborating their pathogenic nature.

#### 4.2. cDNA studies

Knowing that variants that are predictably missense can actually induce splicing defects [14], and since both variants are located in the vicinity of splice sites, we evaluated their possible effects on pre-mRNA processing. Transcript analysis was performed in a cryopreserved muscle specimen from patient 1, using primers designed specifically for the regions of interest. Results showed that neither appeared to have an effect on mRNA splicing (no exon skipping or cryptic splice site activation) and that bi-allelic expression was evidenced with both mutations detected in heterozygosity (Fig. 3).

#### 4.3. Western Blotting

Laminin- $\alpha 2$ ,  $\alpha$ -dystroglycan and  $\beta$ -dystroglycan expression in a muscle sample from patient 1 was assessed by Western Blotting (WB) analysis using monoclonal antibodies against the 80 kDa C-terminal segment of laminin- $\alpha 2$  (MAB1922, Chemicon, Millipore, Temecula, CA), a glycosylated epitope of  $\alpha$ -dystroglycan (VIA4-1, Upstate, Millipore, Temecula, CA) and  $\beta$ -dystroglycan (NCL-b-DG, Novocastra, Leica Microsystems, Newcastle Upon Tyne, UK). The latter

was used as an internal control. The WB procedure was adapted from the protocol reported by Anderson et al. [15]. For the purpose of densitometry, protein loading was normalized with the myosin band in the post-blotted gel. Immunoblot analysis revealed complete absence of merosin with the anti-80 kDa antibody. A 75% reduction was detected in  $\alpha$ -dystroglycan expression by comparison with the control (Fig. 2, H), possibly due to technical issues related with the heterogeneous glycosylation pattern of the epitope recognized by the VIA4-1 antibody.

#### 4.4. Bioinformatic analysis of missense variants

Besides the in vitro experiments described above we also assessed the pathogenicity of the missense variants using a diversity of bioinformatic tools and databases. As depicted in table S1 in supplementary data, all algorithms consistently suggested a deleterious effect for these mutations. In addition, the mutations c.2461A>C and c.812C>T were not present in SNP databanks including dbSNP, 1000 Genomes and Exome Variant Server.

### 5. Discussion

We report two patients with partial laminin- $\alpha 2$  deficiency and atypical phenotypes. The first patient had epileptic encephalopathy with normal muscular examination. The second case had a limb-girdle muscular dystrophy phenotype with unusually severe cardiac involvement. Brain imaging was instrumental for diagnosis. Two missense mutations were found in patient 1, located in the N-terminus of laminin- $\alpha 2$ ; p.Thr271Ile in domain LN (laminin N-terminal) and p.Thr821Pro in domain LEb). Regarding patient 2, two heterozygous mutations were detected: the same missense mutation c.2461A>C

(p.Thr821Pro) in exon 18 and the splicing mutation c.5234+1G>A (p.Val1765Serfs\*21) in intron 36, which has been previously described [2]. The clinical phenotype of both patients is markedly different which could only in part be explained by the different allelic combination of mutations detected in each case. In the Leiden Muscular Dystrophy Pages (<http://www.dmd.nl/LAMA2>), we have found an entry for the c.2461A>C variant, but reported as being of unknown pathogenicity. Interestingly, this mutation was reported in this database in together with the c.5234+1G>A mutation, the same combination as in patient 2. However, the phenotypes of both patients seem to differ significantly (seizures, abnormal white matter, and cervical spine fusion as indicated in the Leiden database, without mention of a cardiac phenotype).

Reports of MDC1A (MIM: 607855) patients with severe cognitive impairment are very scarce and the severity of CNS involvement combined with absent muscular complaints, as described here for patient 1, is a new form of presentation of this disorder. In previous series of patients [7] there was no apparent correlation between mental retardation and severity of weakness, suggesting that different mechanisms contribute to muscular and CNS involvement. Rufinamide was shown to be effective in epileptic encephalopathies other than Lennox–Gastaut syndrome, particularly in patients with drop-attacks and (bi)frontal spike-wave discharges, matching our patient's clinical features [16].

Cardiac involvement in laminin- $\alpha$ 2 deficiency has been largely underreported. One study specifically addressed the cardiac involvement in children with laminin- $\alpha$ 2 deficiency [6]. A cohort of 16 children with congenital muscular dystrophy was studied (6 with MDC1A) where two children with significant left ventricular dysfunction had complete laminin- $\alpha$ 2 deficiency. In another series of 51 patients with MDC1A [4], 15 had cardiac assessment, of whom 5, with complete laminin- $\alpha$ 2 deficiency, had cardiac abnormalities. Documentation on cardiac status was unavailable for the remaining patients. In the largest bibliographical review (248 published patients with abnormal immunohistochemical staining for laminin- $\alpha$ 2) cardiac function was described in 20 [7]. Cardiac dysfunction was reported in 7 patients (7/20, 35%), of whom 4 were asymptomatic. Cardiac abnormalities varied, including right bundle branch block, dilated cardiomyopathy and “borderline changes in cardiac function”.

To the best of our knowledge, only one case of partial defect of laminin- $\alpha$ 2 with cardiac involvement was previously reported [8]. Long-term evaluation led to a diagnosis of dilated cardiomyopathy with ventricular arrhythmias, requiring implantation of an intracardiac defibrillator. In this patient, *LAMA2* gene analysis revealed two different mutations, a missense mutation in exon 29 (c.4405T>C, p.Cys1469Arg) and a nonsense mutation in exon 31 (c.4645C>T, p.Arg1549\*).

We want to highlight the need for cardiac status assessment in this disorder, given the potential of severe cardiac involvement, even in patients with residual expression of the laminin- $\alpha$ 2 chain.

This report widens the clinical presentation associated with genetic defects in *LAMA2*. These cases are probably under-diagnosed and seldom reported in the literature, especially when only subtle changes in laminin- $\alpha$ 2 chain are detected in IHC studies. Considering the interplay role of laminin- $\alpha$ 2 in the basal membrane and extracellular matrix, it is conceivable that mutations in this protein may only affect the interaction with other proteins maintaining at least partial interaction with  $\alpha$ -dystroglycan, and leading to other phenotypes not directly related with muscle weakness.

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.nmd.2014.01.004>.

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